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14. ABSTRACT:

A new series of crosslinked hydrophilic polymer gels based on poly(ethylene oxide) was prepared. These membrane materials showed high water uptake and excellent resistance to bovine serum albumin (BSA) fouling. By controlling the crosslink density of the polymer gels, we were able to manipulate water uptake over a very broad range, from less than 100% to more than 500%, which provides a large window for tuning the permeation and rejection capabilities of these materials. Permeation properties of thin-films made of these gels is also reported. Approximately 20 m^2 of chitosan composite membrane were prepared at our industrial partner, Membrane Technology and Research (MTR). Some samples from this membrane were modified to incorporate the following enzymes into the chitosan thin-film: (i) pronase, (ii) chymotrypsin, and (iii) lipase. The permeation properties of the enzyme-modified composite membranes were tested with an aqueous solution containing either a protein (BSA) or a fatty acid (trilaurin).

15. SUBJECT TERMS
Poly(ethylene oxide) coatings, chitosan, composite membranes, enzymes, memzymes

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Overall Objective: To investigate and optimize strategies to combat organic fouling of polymeric-based membranes in water purification applications using biocatalytically-active nonporous polymer thin-film coatings.

Approach: A systematic investigation involving the optimization of: 1) hydrophilic, crosslinked polymer thin-film coatings, 2) preparation of thin-film composite membranes, 3) enzyme selection, and 4) enzyme immobilization chemistries to polymer coatings, was completed. Characterization of the biocatalytically-active membranes (termed "memzymes" herein) and evaluation of memzyme permeation properties were also studied.

Accomplishments:
1) *Investigate hydrophilic polymer thin-film coatings*

We prepared a new series of polymers to serve as nonporous membrane coatings. Our earlier SERDP and ONR-sponsored work demonstrated the efficacy of PEO-containing materials to reduce fouling. In the earlier work, we used block copolymers containing PEO as the soft, water permeable block and polyamides as the hard, water-impermeable phase. Polymers with higher and, therefore, more optimal concentrations of PEO have been prepared for this program. Poly(ethylene glycol) acrylate is polymerized via standard free radical polymerization (using 0.1 wt% hydroxy-cyclohexyl phenyl ketone (HPK) as the photoinitiator) protocols to prepare a high molecular weight polymer. To obtain better mechanical strength in the final polymer, a crosslinker, poly(ethylene glycol) diacrylate (PEGDA), was added to the initial polymerization mixture. Studies were performed to determine the effect of PEO chain length in the crosslinker on water uptake and water concentration in the initial polymerization mixture on water uptake.

Water uptake increased as the number of PEO units in the crosslinker increased. Crosslinked PEGDA films with Mw~258 (3 PEO units) exhibited a water uptake of approximately 5 wt.%, films of PEGDA Mw~575 had water uptake of approximately 40 wt.%, and films of PEGDA Mw~700 had water uptake of 55 wt.% because PEGDA containing more PEO units is more hydrophilic than that with fewer PEO units. In fact, PEGDA with Mw~258 was sufficiently hydrophobic that it could not be mixed with water; the other two crosslinkers were miscible with water. Therefore, these crosslinkers were used for further investigation.

Water uptake increased significantly with an increase in water concentration in the initial polymerization mixture for PEGDA and PEGA/PEGDA films. Furthermore, free-standing films containing PEGA monomer showed slightly higher water uptake than those prepared using only PEGDA crosslinker. This effect is most likely due to the lower crosslinking density of films containing PEGA. Water uptake values ranged from 40 wt% (100% PEGDA, Mw~575 with no water in the pre-

polymerization solution) to approximately 500 wt% (80 wt% water, 10 wt% PEGA, 10 wt% PEGDA, Mw~700 in the pre-polymerization solution). This result is reasonable because water-polymer interactions decrease crosslinking density during the polymerization process, therefore the more water that is present initially, the less is the crosslinking density and the higher is the water uptake.

Water flux through these films increases as water uptake increases. A PEGDA Mw~700 film made with 80 wt% water in the prepolymerization solution (375 % water uptake) has a flux ten times greater than that of a similar film made with only 60 wt% water in the initial solution (175 % water uptake): 0.22 L m^{-2} h^{-1} for the 80 wt% water film and 0.02 L m^{-2} h^{-1} for the 60 wt% water film at similar operating conditions (30 psig pressure difference, 1420 μm thick film). Both films exhibited exceptional resistance to bovine serum albumin (BSA) fouling and high BSA rejection. No fouling was seen on either film or on a film made with 10 wt% PEGA/10% PEGDA/80% water in the prepolymerization solution. Rejection was above 93% for all films tested (rejection was measured by solution UV absorption at 290 nm).

Enzymes to degrade foulants can be incorporated into a crosslinked PEO matrix using a variety of chemistries. We probed one of these chemistries by introducing a small amount (2 wt%) of glycidyl methacrylate into a poly(ethylene glycol) dimethacrylate film. Enzyme attachment was accomplished using a simple nucleophilic addition between the free-epoxy groups in the film matrix and the lysine residues on the enzyme surface. It was also qualitatively shown that these enzymes (lipase from porcine pancreas and thermomyces lanuginosa) retain activity upon attachment to the film.

2) Preparation of thin-film composite membranes

During this study, 20 m^2 of nonporous chitosan thin-film composite membrane was made on MTR's commercial coating equipment. Samples were sent to Professor Clark's lab at the University of California at Berkeley for incorporating enzymes in the membrane.

The membranes to be developed in this project consisted of (i) a microporous support and (ii) a nonporous polymer coating containing an enzyme or a series of immobilized enzymes. During this period, MTR made membranes based on a microporous poly(vinylidene fluoride) [PVDF] support and a thin, nonporous crosslinked chitosan layer. The polymer was puchased from Aldrich Chemical Company (Milwaukee, WI). The composite membrane was made by a continuos dip-coating process on MTR's commercial 40-inch-wide coating equipment. The composite membrane was made by coating a 0.3 wt% solution of the polymer in a 1:1 mixture of water and ethanol onto a microporous PVDF support. Glutaraldehyde was used as the crosslinking agent for the chitosan membrane. The polymer to crosslinker ratio was 50:1. The membranes were dried in an oven at 100°C.

3) *Enzyme selection and enzyme immobilization chemistries to polymer coatings.*

We have immobilized enzymes onto nonporous chitosan-coated membranes and measured the hydrolytic activities of the resulting memzymes. Two

proteases and one lipase were selected (α-chymotrypsin, pronase, and Lipase PS). α-Chymotrypsin from bovine pancreas is specific for aromatic residues, and pronase from Streptomyces griseus is unusually non-specific. Lipase PS from Burkholderia cepacia was reported to exhibit the highest activity toward p-nitrophenyl palmitate among 32 commercial lipase preparations. Lipase PS also turned out to have the highest activity toward a glycerol-derived substrate (1,2,-O-dilauryl-rac-glycero-3-glutaric acid-resorufin ester) of 4 lipase candidates (Lipase PS, Lipase AK from Pseudomonas fluorescence, Lipase AY from Candida rugosa, and Chirazyme from Candida antarctica). Our initial enzyme immobilization work has focused on the hydrophilic chitosan composite membrane which provides amino groups for immobilization of enzymes to the chitosan surface.

To this end, chitosan membrane was incubated in a sodium borate buffer (50 mM, pH=10.0) with or without 1% glutaraldehyde (GA) as a protein coupling agent at room temperature for 5 hrs, and then washed with distilled water. Enzymes were immobilized to the membranes by combining enzyme solution (1.0 mg/mL in 50 mM sodium borate buffer, pH=8.5) with activated or non-activated chitosan membrane at 4°C for 24 hrs. The resultant membranes were washed with 1 L of 50 mM sodium acetate/0.5 M NaCl, pH 5.0, and 1 L of 50 mM sodium borate/0.5 M NaCl, pH 9.0 (alternating 500 mL of each), and finally with 1 L of 100 mM sodium phosphate, pH 7.0. Catalytic activities of the memzymes (α-chymotrypsin, pronase, and Lipase PS) were determined spectrophotometrically with suc-ala-ala-pro-phe-*p*-nitroanilide, L-leucine-*p*-nitroanilide, and *p*-nitrophenyl butyrate, respectively. Memzyme activities without or with GA activation are shown in Table 1. Additional GA treatment (activation) did not enable higher enzyme activity/loading for either α-chymotrypsin or Lipase PS; however, in the case of pronase, the activity was significantly higher for the membrane treated with GA.

Table 1: Activities (nmol s^{-1} cm^{-2}) of various memzymes

Type of memzyme	α-chymotrypsin	Pronase	Lipase PS
Without GA activation	0.095 ± 0.02	0.2 ± 0.05	0.24 ± 0.06
With GA activation	0.07 ± 0.03	0.12 ± 0.01	0.26 ± 0.005

In conclusion, α-chymotrypsin, pronase, and Lipase PS retained good activity upon attachment to chitosan composite membranes. Enzymes appeared to be covalently attached, with or without GA treatment. Covalent attachment to the non-GA-activated membranes presumably occurs through pendant aldehyde groups on GA introduced during cross-linking of the chitosan in the coating process. However, additional GA treatment enabled a 5-fold increase of pronase activity/loading.

4) Evaluation of memzyme permeation properties

The permeation properties of pure chitosan and enzyme-modified chitosan composite membranes were determined in a cross-flow permeation system over a test period of 10 days. The experiments were carried out at a feed pressure of 120 psig and 25°C. The permeate pressure was atmospheric (0 psig). The feed flow velocity was 0.23 gpm. Pronase- and

chymotrypsin- modified chitosan composite membranes were evaluated with an aqueous feed solution containing 1,000 ppm BSA. Lipase-modified chitosan composite membranes were tested with a solution containing 500 ppm trilaurin and 500 ppm Triton X-100. The results are shown in Figure 1.

In the first set of experiments, the permeation properties of chitosan (CH), pronase (PCH)- and chymotrypsin (CCH)-modified chitosan were evaluated using an aqueous solution containing 1,000 ppm BSA. The enzymes were attached to the membrane surface without any additional pre-treatment. During the first 4 days of operation with a BSA/water feed solution, the water fluxes of all membranes dropped to about 50% of their initial pure-water flux values. Thereafter, the water fluxes remained essentially constant for a permeation time of up to 10 days. However, it appears that the enzyme-modified chitosan membranes showed very little or no activity, as their permeation long-term properties were very similar to those a pure chitosan membrane. All membranes exhibited a BSA rejection of 98%.

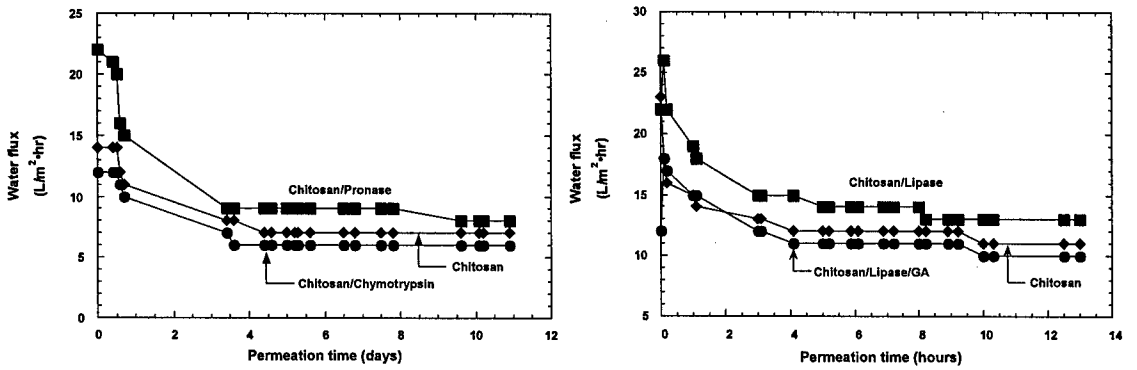

Figure 1. Long-term permeation properties of various composite membranes. Feed: a) 1,000 ppm BSA in water (left graph) and b) 500 ppm trilaurin/500 ppm Triton X-100 in water (right graph); feed pressure: 120 psig; temperature: 25°C; feed flow velocity: 0.23 gpm. Note: Data points at initial permeation time (0 days) correspond to pure water flux.

Conclusions: By controlling the crosslinking density of the polymer gels, we were able to manipulate water uptake over a very broad range, from less than 100% to more than 500%, which provides a large window for tuning the permeation and rejection capabilities of these materials. Furthermore, these polymer gels can incorporate enzymes and sustain their activity for extended periods of time. These materials are slated to be the next generation of thin film coatings on the conventional porous membranes being used in this program. During this period, MTR produced about 20 m^2 of nonporous chitosan thin-film composite membrane on their commercial coating equipment. These membranes were sent to Professor Clark at the University of California at Berkeley for incorporating: (i) pronase, (ii) chymotrypsin, and (iii) lipase into the chitosan layer of the membranes. The membranes alone (i.e., without enzyme modification) showed significant resistance to fouling by a model protein (BSA) and a model fatty acid (trilaurin). The fouling resistance of the membranes was not improved significantly due to the presence of the immobilized enzymes.